17 MAR 1983
Dr. Eula Bingham
University of Cincinnati
ML 627
Cincinnati, Ohio   45221

Dear Dr. Bingham:

As the USEPA On Scene Coordinator for the Reilly Tar site I am involved in the design of studies and analysis of alternatives that would effect a cleanup of the site. Stephen Shakman, Assistant Attorney General, State of Minnesota has brought to my attention the recent work conducted by R. E. Lovrien and others regarding mutagenicity testing of Polynuclear Aromatic Hydrocarbons found in contaminated sites including St. Louis Park, Minnesota.

I have attached for your review the above mentioned study and would appreciate your comments relative to the utility of Lovrein's work to your work to be conducted by you and Dr. Selkirk at the Reilly site. I am, by copy of this letter, requesting Dr. Selkirk's review and comments.

If you have any questions please call me at (312) 886-3007.

Sincerely yours,

Paul Bitter
On-Scene Coordinator

cc: Selkirk, Oak Ridge National Lab
      Kasakowski, WH527-E
      Shakman, MPCA; AG
      Leininger, 5RC
      Hird, DOJ

bcc: M. O'Toole, RRS II, w/copy of study (attached)
      K. Waldvogel, RRS II, w/copy of study (attached)
      J. Schulteis, 5RC, w/copy of study (attached)

: Mike :

This is a first report on the St. Louis Park
drill sample, which we looked at via A series
mutagenicity testing. (Not all copies here, but
you can pick an action).

Mutagenicity of Twin Cities and Minnesota Chemical Dumps: Mutagenicity's Bearing on Chemical Carcinogenesis and Human Risk Assessment in Reference to T. C. Campbell's Review

R. E. Lovrien, T. Wilson, and E. G. Schanus

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August, 1982

Main point is that some of the St. Louis Park
samples are _extremely_ mutagenic; Some are far
more mutagenic than anything we've seen in
all the direct experiments mutagene assay
action.

Two of the three largest chemicals dumps in the Twin Cities are dominated by coking chemicals. Coking chemicals are of unusual concern: Coking chemicals are among the most mutagenic, biohazardous, procarcinogenic of general classes of industrial chemicals. Coking chemicals are also toxic (cytotoxic). Cytotoxicity towards cells affects the course of mutagenicity assays. Estimation of the interplay between mutagenicity and cytotoxicity can be made in two ways: (i) By means of mammalian enzymes known as liver microsome S9 enzymes. The S9 enzymes are used as standard parts of the Ames in vitro procedure for expressing the tendency of liver enzymes to activate chemicals to procarcinogens. (ii) By separation of such mixtures into fractions, having varying tendencies to be toxic on the one hand, mutagenic on the other, and aligning that information with chemical analytic information. This is the first report based on our first measurements on Kopper's site samples. Some of the points will be amplified later, and data on the St. Louis Park dump will be described. Our notation is K = Koppers (K_1 = sample no. 1 from Koppers), S.L. = St. Louis Park, C = Control soil and water samples, PAH = polyaromatic hydrocarbons and DPAH = derivatized and rearranged such hydrocarbons.

The major points are:

(1) Large tonnages in the Koppers dump are coking chemicals.

(2) Coking chemicals were used in the Republic Creosoting dump in St. Louis Park, evidently with some redistilling. Later, pentachlorophenol(s) were used as additives.

(3) Raw extracts from the K_1 samples have specific mutagenicities in Ames testing which rank them as equal to or closely approaching the promutagenic potential of some of the more biohazardous, established "PAH" compounds.

(4) There is little surprise, concerning point (3). It has been known for decades, a century in fact, that coking and soot chemicals are among the most procarcinogenic of industrial agglomerate mixtures. Part of the reason is that coking chemicals are not simply mixtures of "PAH" (Polyaromatic hydrocarbon) compounds. Coking chemicals tend to be derivatized PAH compounds, (DPAH) mixtures of purely PAH plus larger mixtures of many kinds of nitrogen and sulfur derivatives of PAH, often partially oxidized, phenolics, etc. Many of such coking chemicals tend to be heterocycles. In fact, Reilley Chemical Co. of Terre Haute has its major markets in aromatic nitrogen heterocycles, pyridines and quinolines, etc. which is likely one of the reasons why Reilley bought the coal tar plant.

Local reports have sometimes dwelled on a comparison of cooked meats' erstwhile biohazard potential, basically asking the question: 'If one is not afraid to eat a cooked hamburger or two, why worry about the water? Both have about the same PAH content'. If simplified questions such as this are to be asked, one may inquire about the ancient - but probably still valid - data concerning testicular cancer in chimney sweeps. Coking chemicals are much more closely related to soot and to benzidine than they are to cooked hamburger. Perhaps topical or cutaneous applications of procarcinogens cannot be compared with ingested application. But detailed arguments in this vein are unprofitable. In the meantime, the best stance may be to be conservative - but in both directions. Maybe our coking chemicals are not so comparable to the coal tars in the soot of English chimneys. On the other hand, comparisons of cooked hamburger with our coking chemicals is not an especially illuminating comparison, either, despite the erstwhile studies and reports by our local agencies.

(5) Why has there been so much emphasis on "PAH"? There are two main reasons: (i) PAH are indeed important, even though PAH may not be of surpassing importance in coking chemicals, relative to the DPAH. (ii) The PAH compounds have been convenient to analyze and are the most discussed surrogate compounds for several classes of industrial chemicals, particularly petrochemicals. Rosenkranz (Mutation Research 101, 1-10 (1982)) points out that it is becoming clear in the case of diesel exhaust hydrocarbons, that the early concentration on PAH occurred because of philosophic considerations from several directions, combined with a lack of any comprehensive measurements. It is now seen that the potent mutagens in diesel exhaust are nitro- derivatives of PAH compounds. To be sure, BAP (benzo[a]pyrene) and other PAH are present, but the nitrogenous derivatives are those needing the most focus. The same is likely true in coking chemicals, since part of the pyrogenic process in coking fixes nitrogen. The black color of coal tar derivatives stems from oxidized nitrogen aromatics and their condensates with phenols which coal tar is also rich in. Purely PAH compounds are not black, they are white, or colorless. Thus a strong hint as to what coking chemicals may be, is gotten by simply looking at them. As Casey Stengel once said, 'Sometimes you can observe 'tings, just by lookin''.

There are two further reasons for the concentration on PAH: (iii) Once an agency starts a program and issues reports, it may be reluctant to consider much change in emphasis. (iv) Most GC-MS (Gas chromatography-mass spectrometry) methods are set up and have been calibrated for analysis of PAH. The nonchemist, and agency administrators, may be easily impressed by the power and sensitivity of GC-MS methods. But that does not mean that such "analyses" have told the whole story at all, or even a large fraction of it. Neither the EPA, nor any other agency, have said that it is a good idea to exclude all other classes of compounds in such mixtures, to make it appear that PAH compounds should receive exclusive focus. It is time to start getting realistic about these mixtures. The GC-MS "PAH analyses" give some information - maybe. But in coking chemicals, that information is likely to be peripheral. The GC-MS PAH analyses may apply quite well to some petrochemicals, perhaps to nonpyrolytic coal chemicals. Agreed, they may apply to certain compounds in the EPA priority pollutant list. But whether they apply to coking chemical DPAH is likely questionable, until such is really shown.

(6) The DPAH compounds are vast mixtures of compounds derivatized in dozens of ways, with heterocyclics, aza-linkages, cyano, nitrile, phenolic and quinonic groups. Purely primary amine derivatized DPAH compounds may be lesser in quantity, since they are more fragile toward oxidation than several other classes of DPAH. The substituents on DPAH are particularly likely to enhance their water solubility, and it needs be remembered that coking pyrolysis also produces many acids which are likely to protonate nitrogen DPAH. In the movement of DPAH downward, sometimes it is assumed that deep ground waters are deficient in oxygen. However, recent, careful measurements of such ground waters, 300 to 3000 feet down by Winograd and Robertson (Science 216, 1227-1229 (1982)) are contrary to the common assumption. Frequently deep ground waters run from 4 to 6 mg. O_2/liter, not much less than surface waters. One expects that deep ground waters in our local dumps might be depleted, because of the large amount of reducing compounds in the soils. But some continuing oxidation, and therefore movement and solubilization is likely. One notes that a number of supposedly carcinogenic PAH compounds are not, per se, carcinogens. They are only procarcinogens. It is their partly oxidized derivatives which are actually active carcinogens.

(7) The Ames mutagenicity data we gathered with the K_1 sample (raw, whole extract) is attached here. The same techniques and protocol for most of the work, which we have been using for three years on diesel exhaust compounds were used here.

The main point is that the K_1 sample is powerfully mutagenic. In the Ames organisms which mutate from benzo[a]pyrene (BAP), for instance, on a specific or per weight basis, simply the raw Koppers soil sample is at least 10% as mutagenic as pure BAP. When it is considered that the K_1 sample is a vast mixture, containing ingredients which may not even be soluble in the solvents we have to use to dose the organisms, and that most likely many compounds are inert, one fairly confidently predicts that fractions of K_1 can be split out that shall be much more mutagenic than even BAP.

The measurements for the data attached here were preceded by a set of preliminary experiments known as spot tests. These are done for two reasons: First to determine the best dose range, and second to see if and how cytotoxicity affects the mutagenicity assay. It was quickly apparent that cytotoxicity is very important in K_1 samples, but that the mutagenicity assays can be carried out in extreme low dosage ranges (before the organisms are killed).

Some idea of the dimensions of the mutagenicity of raw K_1 site sample can be gotten by comparison with diesel particulate extract (DPE) compounds. Usually a dose response slope of 0.5 to 1.0 for most strains (the units on these slopes are specified below) is cause to call them quite biohazardous, and slopes of the order of 1 to 5 are fairly likely to be carcinogenic. It is now known in diesel compounds, dose reponse slopes of that kind are often dominated by nitroaromatics (Salmeen et al., EPA Diesel Emissions Symposia (1981), Schuetzle et al., Intern. J. Environ. Anal. Chem. 9, 1-52 (1981)). The most mutagenic diesel extract we have ever seen in our laboratory, on Ames strain TA100, had a slope of about 42. This kind of diesel extract descends from experiments in which the diesel exhaust was deliberately dosed with large levels of both ozone and $(NO)_x$ (nitrous oxide mixed gases), plus u.v. irradiation to create the most biohazardous conditions possible. As for the K_1 raw extract dose response slope, with the TA100 strain, (+)S9 activation, we got a slope of 45 (1).

The next items review our measurements in more detail, and make further comparisons. There is no doubt that the K_1 sample is mutagenic, in the extreme. The meaning of that, in human biohazard potential vis a vis these short term assays, needs be considered in context with a number of current reviews, e.g. the Hollstein and McCann, or the T. C. Campbell review. The references are given below.

(8) The detailed protocol for performing Ames mutagenicity testing is rather lengthy (10 pages of single space type). It will be sent to anyone needing it. Our protocol is in the hands of the EPA, the CRC, a number of companies (Calspan, Ford, GM, Caterpillar, and Exxon). It is mainly a detailed repeat of the procedures described by Ames and collaborators of the Univ. of California, Berkeley laboratories. E.g. J. McCann and B. Ames, Origins of Human Cancer, in Hiatt et al., eds., Cold Spring Harbor Laboratory, New York (1977), 1431-1450; Ames, B., McCann, J. and Yamasaki, E., Mutation Research 31, 347-364 (1975); McCann, J. and Ames, B., Proc. Nat. Acad. Sci. 73, 950-954 (1976). The changes we advocate with respect to the Ames et al. protocols have to do with increasing the precision of the assay, and methods of prevention of what are known as star colonies. We also produce our own (+)S9 mammalian microsomal enzymes (Arochlor 1234 induced rat liver), rather than purchasing it, and calibrate the (+)S9 preparations.

(9) We now report our first organized mutagenicity measurements of the K_1 sample taken at the surface of the soil, between the B.N. railroad, the coal elevator, and the "devil's liquor" dump sector of Koppers. Four of the five principal tester strains were used, TA100, TA98, TA1538 and TA1537. The nature of the mutations (base pair substitution, frameshift, etc.) detected by the procedures is described in the Ames, McCann, and Yamasaki paper.

Our values are:

Strain	Mammalian enzymes	Slope (initial slope) in Revertants/microgram	Intercept	Correlation
TA 100	(+)S9	45.5	172	1.00
	(-)S9	(-) 1.2	143	0.38
TA 98	(+)S9	13.3	55	0.87
	(-)S9	5.1	44	1.0
TA 1538	(+)S9	6.9	16	0.98
	(-)S9	0.5	15	0.40
TA 1537	(+)S9	8.2	20	1.00
	(-)S9	0.4	9	0.96

At the same time, a check was run using benzo[a]pyrene (BAP) with TA100, which is activated with (+)S9. We obtained a slope of close to 430 Rev./µg, and Ames et al. quote 460 Rev./µg. Our strains were all checked immediately beforehand with the compounds and methods used for genetic fidelity checks of the strains. These are described in the more detailed protocol, and again, are necessary checks in work with the Ames S. typhimurium tester strains.

We left out TA 1535 here because we were running diesel samples at the same time which were rather scanty in size. In order to economize in sample, the choice was to leave out TA 1535. In the future, with Koppers, also St. Louis Park samples, obviously TA 1535 should be included.

(10) Some features of the data, and the rough plots we made, are necessary to note.

First, the intercepts on the plots for each strain should agree reasonably well (±30%) with what are fairly well accepted average values for the natural revertants (NR) case of zero dose, and they do. We may be a little high with TA 1537 in the (+)S9 case, but by and large our numbers of NR for each strain, and their values in comparison to the intercept values, are in good agreement. The numbers of NRs for the individual strains gotten by Ames et al. are published by them in several places.

Second, the dose response slopes, one of the most important quantities, are the initial slopes, independent of the intercept. It is important to show both. It is also important to include all raw data, so that the user can replot, if wanted. The raw data are included here, as part of our protocol. The plots as drawn are only suggested plots, which issue from the way our plotter is programmed. Again, users of the raw data may well want to replot, using for example smooth curves instead of discontinuous straight lines connecting the points. Despite the slightly arbitrary way of plotting our data, however.

is certain that we have here compounds which are mutagenic in the extreme, and that (+)S9 enzymes are necessary to show that.

It also seems certain that besides extremely mutagenic compounds, we have here extremely toxic compounds which kill the microorganisms. It is very likely that it is bacterial kill which sends the (-)S9 plots downward - precipitously downward in many cases, even producing negative slopes. Of course, once killed, such bacteria cannot mutate and hence produce no visible colonies.

Thus the sharp, high slopes with (+)S9 may not be exclusively a matter of mammalian enzyme activation. Rather, the (+)S9 enzymes may help detoxify, by unknown mechanisms, or otherwise help keep the organisms alive so that they can mutate. There is no reason at all to think, therefore, that the upper limits of mutagenicity are seen even with (+)S9. Cytotoxicity likely occurs with (+)S9, and we clearly see that the (+)S9 plots lop over, i.e. flatten, even go downward to the abscissa, out in the finite dosage range. Such is a well accepted indicator of cytotoxicity. Thus that which is seen is a matter of two forces, as the dose increases: (i) Mutation, perhaps activated by (+)S9, and not activated in the case of (-)S9. (ii) Cytotoxicity, increasing with dose, modulated some by the S9 enzymes. but not preventable with the larger doses.

Finally, it is necessary to note the scales, on both ordinate and abscissa, if these data are compared with other laboratories. We are operating here with very small doses, partly because of the toxicity problem, partly because of the violent mutagenicity coupled with the need to get initial slopes. In sum, very small doses of the Koppers compound are potent, in the extreme, in both respects.

(11) Evidently work on the Koppers site and St. Louis Park sites needs to go forward. We shall also obtain a number of control samples, but it is unlikely that soils in the rural area, or even most urban soils, possess nearly the mutagen density of the chemical dump soils. Particularly in the case of coking chemical. There is also a distinct possibility of synergism between compounds like pentachlorophenol and cooking chemicals, i.e. that they may enhance one another's biohazard nature.

What are the practical aspects in human biohazard? We or others shall have to determine soil densities of such compounds, rates of washthrough and hydrologic channeling, solubilities and other parameters to get a complete picture. Data in these areas is rather smaller than the size of the problem(s).

In human biohazard potential, we would tend to rely on the analyses of, for example, T. C. Campbell.

(12) The Koppers K_1 sample (and during the last few days, the St. Louis Park SLP deep well samples gathered at the Renner drilling rig site) are mutagenic in the extreme. They are profoundly mutagenic even without fractionation. Probably fractions can be split out, of larger - and probably of smaller - mutagenicity than the average which we see now. We are in the process of obtaining organic nitrogen contents.

The bearing of mutagenicity on human biohazard merits careful attention. Here, we shall not try to write a review. Instead, we refer to three reviews concerning environmental mutagens and carcinogens, and quote from one such review. Namely: T. Sugimura et al., eds., "Environmental Mutagens and Carcinogens", Third Internat. Conf. Concerning Mutagens (1982), Liss Pub. Co., N.Y.;

M. Hollstein and J. McCann, Mutation Research 65, 133-226 (1979); T. C. Campbell, Fed. Proc. 39, 2467-2484 (1980).

Three pages of Campbell's review are copied and attached. They illustrate why, beginning about 1977, arguments that bacterial mutagenicity tests, Ames method in particular, have no predictive powers in mammalian carcinogenicity, became quite untenable arguments. It may never be proven with 100% certainty that all intensively mutating compounds always predict mammalian carcinogenicity. But the weight of all the data which has been reviewed can hardly be tossed aside now. Dosage ranges over which tumor induction correlate convincingly with the Ames test (and with other kinds of assays, reviewed in much detail by Hollstein and McCann). The dosage ranges traverse nearly six orders of magnitude, 10^6 fold, and include hundreds of compounds. It is noted in the case of coking chemicals, where the aminoaromatics fall in such correlation plots. Note the position of benzidine and 4-aminobiphenyl in Figure 2 of Campbell's review, which in turn is from Meselson and Russell's paper. In our K_1 sample, and in the St. Louis Park samples ((+)S9, TA100), in which we measure slopes of 45 and of 10 Revertants/microgram/plate, we have that we require about 2 to 10 micrograms per 100 Revertants (above spontaneous revertants), to put our data on the same scale as the Meselson and Russell plot. Hence the raw coking chemicals, even without fractionation, fall about in the middle of the Meselson-Russell (Campbell) plot. We are not certain that all our K_1 and SLP samples were truly dissolved, and if they were not, then the compounds are even more mutagenic than that which we currently estimate.

In 1976, McCann and Ames ("Origins of Human Cancer", Hiatt et al., eds., Cold Spring Harbor Laboratory Conferences, p. 1440) wrote, quote: "The Salmonella mutagenicity test is currently detecting about 90% of organic carcinogens as mutagens. We expect that with further improvements in both the tester strains and the metabolic activation system as discussed above, the test will detect at least 95% of all carcinogens. ...The remaining few precent will never be detected by the Salmonella/microsome (S9) bioassay". Unquote. Hence one sees, from Campbell's review four years later and Sugimura et al. six years later, how well the Berkeley workers' predictions came true.

(13) It is necessary to carefully regard not only the mutagenicity of the K_1 and the SLP samples, but also their cytotoxicity. One gets a foretaste of what might happen to Ames bacteria, from the appearance of these dumps, where even weeds do not grow nearly 10 years after they were shut down. Since these samples kill microorganisms so readily, being relieved only by (+)S9 which enables the microorganisms to stay alive long enough to mutate and start reproducing, cytotoxicity is most likely to operate in a direction such that we shall underestimate mutagenicity. Thus with (-)S9, the organisms are wiped out so thoroughly by K_1 and SLP chemicals that we simply get back apparently negative slopes. The function of (+)S9 here is not simply to act as a surrogate or replica of mammalian enzymes, but also as a detoxification agent. Thus, even if one sidesteps the issue of mutagenicity, there is no doubt that the raw extracts of K_1 and SLP are profoundly cytotoxic.

(14) Some idea of the dimensions of mutagenicity of the raw Koppers site sample (K_1 sample, tarry surface deposit) can be gotten by comparison of the dose response slopes we get, the dose response slopes for standard promutagens, and of diesel particulate extract compounds which provide the main background of our experience. We quote below from a 1982 paper by Rosenkranz, who reviewed the diesel compound mutagenicity from a number of laboratories.

For Benzo[a]pyrene (BP), McCann et al. (McCann, Choi, Yamasaki and Ames, Proc. Nat. Acad. Sci. 72, 5135-5139 (1975)) quote 2398 Revertants per 5 micrograms per plate of TA100, (+)S9, or a slope of 480 Rev./μg of B.P. We repeated these a number of times, and usually got close to 420 ± 30 Rev./μg in our earlier work (N = 5) and lately we get 440 ± 30 in N = 4 separate determinations. Attached is a plot showing such data, also the number of natural revertants (NRs) which we usually get.

The initial slope for the Koppers site raw sample, K_1, for TA100, (+)S9 is 45 Rev./microgram/plate. I.e. simply the raw sample is 10% as mutagenic with TA100, as pure BP.

Another comparison might be made by noting the mutation of TA100 by the most violently mutagenic diesel samples which we have ever recorded. Namely, samples in which the diesel exhausts were subjected to ozonolysis and ultraviolet light, a process known to greatly enhance diesel biohazard, for several hours after emission. From the ozonided diesel raw extracts, we obtained 46 Rev./μg/plate (TA100, (+)S9), fortuitously close to BP's value. In the SLP samples, we are using as a check compound 7,12-dimethylbenzanthracene (DMB), in addition to BP, and will report the slope for DMB on the data sheet.

Rosenkranz (Mutation Res. 101, 1-10 (1982)) cites several laboratories using strain TA98 to show that one obtains, with nitropyrene dosing of diesel raw extract, 93 μg of nitropyrene/gram of diesel extract, a value of about 270,000 Revertants/gram extract. Converting to our units, about 0.27 Revertants/microgram. In our Koppers K_1 sample for TA98, (+)S9, we obtained 13.3 Rev./microgram. Thus the K_1 raw extract may be about 13.3/0.27 or about 50X as mutagenic as the nitropyrene dosed sample discussed by Rosenkranz. (It is the interplay between atmospheric nitrous oxides, ozone, sunlight, and combustion exhaust from both spark ignition and diesels, that are the basis for concern in areas such as the Los Angeles basin. It is almost certain now that it is the nitroaromatics which are the most biohazardous, mutagenic and procarcinogenic, fractions of diesel and other exhausts).

(15) We are aware that such comparisons can be misleading. The intrinsic biohazard nature of compounds in laboratory assays do not tell us anything about how the compounds are distributed. In the Koppers and St. Louis Park sites, water solubilities, hydrologic throughput, and soil adhesion are factors. We will defer comments on those for a later report. But we note here that nitrogen derivatized aromatics are almost certain to make for larger water solubilities than the parent pure "PAH" compound. The same is true, generally (but not always) for insertion of oxygen in the parent aromatics. That is, as the compounds become derivatized, they become of enhanced mutagenic potential and of enhanced water solubility. After enough oxygen is inserted, to fracture the compounds or allow them to become metabolized, on the way to CO_2 and H_2O, biohazard potential decreases. But in the case of coking compounds, we predict that their derivatization is such that they are nearly at their maximum potential in biohazard potency. Unfortunately, they are so bactericidal and bacteristatic (relatives of antiseptic agents) that they do not get turned over in any soil carbon cycle. Hence they simply lie there. We note for the reader interested in chemicals distribution in soils and water, the J. Smith et al. report from the Stanford Research Institute (Federal publication EPA-600/7-78-074, May (1978)). It is entitled "Environmental Pathways of Selected Chemicals in Freshwater Systems". It is a rather impressive source of solubility data and also as a literature review.

MUTAGENICITY (AMES) ASSAYS FOR

THE K_1 SITE AND S.L.P. SITE
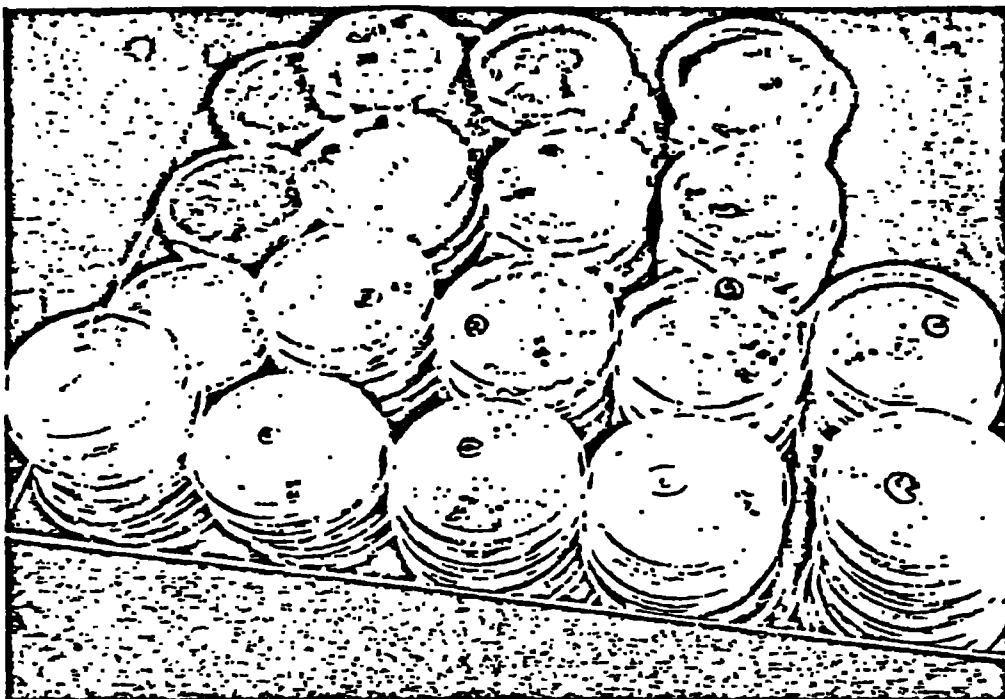
ST. LOUIS PARK
BARREL #7
TA 100

(-) S9   (+) S9
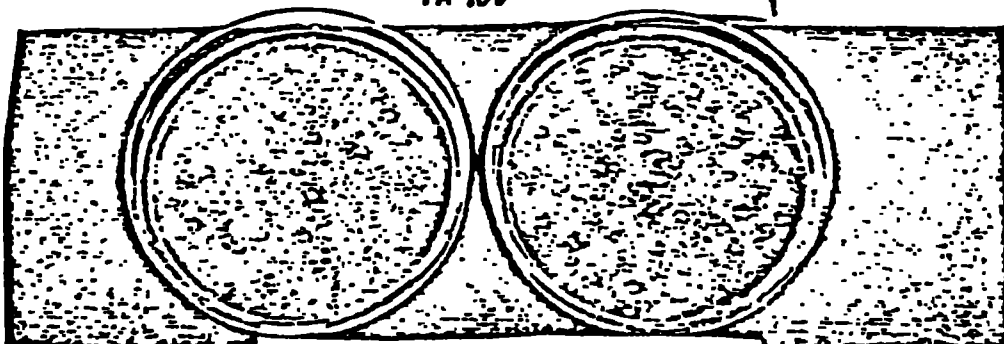
Sample ___Koppers Total Extract.___ Standard compound ___B.P. (Benzo[a]pyrene)___

Bacteria Strain No.	μgr. sample per plate	No. colonies per plate without S-9	Slope/ Corr./ Inter.	No. colonies per plate with S-9	Slope/ Corr./ Inter.	Std. Compd. +/- w/o S-9	w S-9
TA 100	0 5 10 20 40 80 160	119 189 137 130 150 153 95 128 110 164 95 123 86 96 x 43 42 63 141 50 39		155 x 189 408 411 378 518 502 542 621 717 607 601 567 795 788 652 655 190 131 168		175,194, 141	847,89- 907
TA 98	0 5 10 20 40 80 160	48 41 41 60 79 443? 85 x 50 55 47 267 172 39 49 35 45 19 x		x 49 31 126 227 101 x 176 170 284 291 261 262 255 267 295 168 322 272 248 x		44,55, 48	369,55, 421
TA 1538	0 5 10 20 40 80 160	15 12 x 16 36 11 25 14 16 12 20 9 13 12 14 8 12 32 9 7 9		17 17 22 51 32 51 80 95 88 131 134 95 180 174 154 207 195 212 215 248 211		18,21, 10	196,193. 169
TA 1537	0 5 10 20 40 80 160	5 13 8 9 16 9 12 12 14 4 13 x 12 8 5 5 2 7 3 9		16 18 52 69 62 67 82 90 120 110 123 120 111 141 92 94 106 86 99 97		12,11,9	129,11?, 126
TA 1535	0 10 20 40 80 160						

Comments: _x and blanks = star colonies; not plotted._

Keppers, S.. 12
Strein 1538

INITIAL SLOPE = 6.90

(+) S9

(-) S9

Recombants per plate

280
240
200
160
120
80
40

20   40   80   160

mg per plate
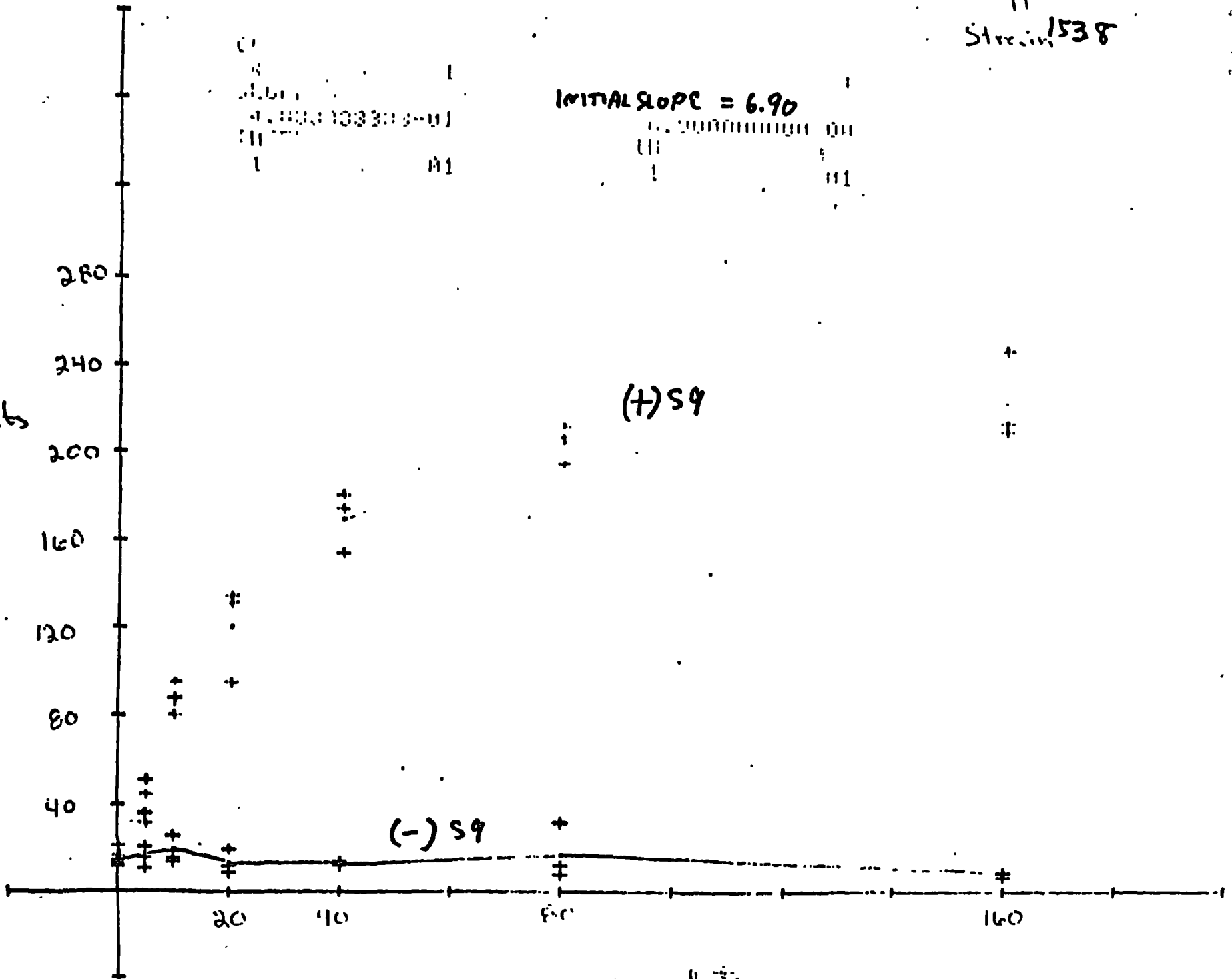
Purchase et al. (34) used a battery of three mammalian cell lines (human lung, human liver, baby hamster kidney) to test for cell transformation, which has been reported to be a result of mutational damage (20). They found that 91% of the animal carcinogens were mutagenic in this system; this agreement was identical to that for the Ames test (91%). When the accuracies of prediction for both carcinogens (91%) and noncarcinogens (97%) were combined, the mammalian test was 94% accurate and compared with 93% accuracy for the Ames assay. The mammalian test was found to produce 3% false positives and 9% false negatives; perhaps the additional criterion of initiation activity would allow these data to approach 0%, as with the Ames assay. An important result of these studies was illustrated with the finding that when carcinogens were judged to be mutagenic by a positive response in either one or both tests, 99.19% of the carcinogens were mutagenic! Thus, use of both tests improved predictability to near perfection. With this same criterion, there were still only 8.8% false positives; once again, simultaneous demonstration of initiating activity should reduce this latter "error."

The conclusion that these tests, used independently, are correct 93–94% of the time (34), and when used collectively, 99% of the time, certainly makes a very persuasive argument in favor of their utility. The added power that should be contributed by the demonstration of initiating activity (covalent adduct formation) might make these systems as accurate as ever can be achieved. Incidentally, it should not be forgotten that any observed relationship is only as reliable as are the results of the animal tests. If controversially carcinogenic compounds such as phenobarbital, dieldrin, DDT, saccharin, xylitol, and sucrose are considered, their improper assignment would become an obvious source of error. Moreover, as Ames has stated, the sensitivity of animal tests is an additional experimental parameter that must be considered in this type of analysis.

Relative carcinogenic and mutagenic potencies

Another characteristic that must be considered when evaluating the utility
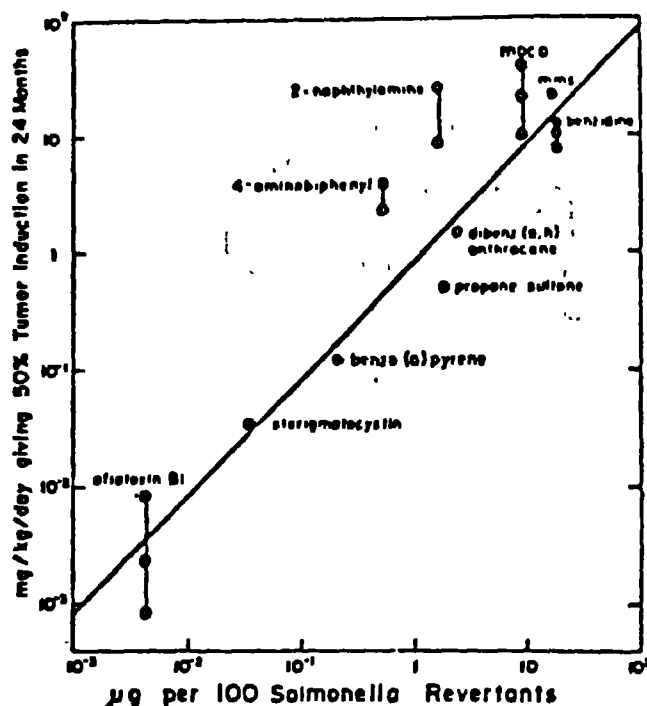

Figure 2. Relation between mutagenic and carcinogenic potency (Meselson and Russell (29)).

of the short-term test is the relative carcinogenic and mutagenic potencies (A to B, quantitative relationship). Meselson and Russell (29) have published a fascinating study on this point. They calculated the animal carcinogenic potency[3] for a group of 10 chemicals and compared this to their mutagenic potencies[4] in the Ames test. Their results are shown as a log-log plot in Fig. 2, in which the ordinate represents carcinogenic potency and the abscissa mutagenic potency. Furthermore, they suggested that carcinogenic potency for man may be roughly equal to that in rodents if the carcinogenic potencies are expressed in terms of the average normal lifespans of the respective species (B to C, quantitative). They further considered that if the slope function for man is not too different from that of rodents, then carcinogenic potency for man (expressed as a lifetime probability) might be directly estimated from the mutagenic potency in the Ames test (A to C, quantitative relationship).

These data would therefore suggest the emergence of reasonably impressive correlations of potencies both within selected in vitro mutagenesis assays and between mutagenic potency in the Ames test and carcinogenic potency in animal tests (A to B, quantitative). Nevertheless, most reports on the subject have cautioned against predicting human carcino-

genic potency either from a single mutagenesis assay or even from a battery of assays (A to C, quantitative). This was first noted by McCann and Ames (27) and more recently in two publications by Ashby and Styles (5.6).

The conclusions drawn from these latter discussions on the correlation of mutagenic potency with carcinogenic potency (in any of multiple animal species) are varied, suggesting that the relationship is "approximately equal" (words of Meselson and Russell (29)), "good" (word of Ames and Hooper (3)) or, perhaps, "superficial" (word of Ashby and Styles (5)). Specifically, Meselson and Russell (29) showed a range in mutagenic potency of 10^4 for their 10 chemicals and Ashby and Styles (5) claimed a variation for an individual chemical to be 10^2. On the other hand, Ames (1) cites a 10^6-fold range in the mutagenic potency for all chemicals. In any event, variance for an individual chemical of 10^2 does not seem large (0.01 to 1%) when compared against the mutagenic potency range for all chemicals, and thus, does not violate

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[3] Carcinogenic potency was calculated as the daily dose that gives 50% cumulative single-risk incidence after 2 years of exposure, which is the average life span of rats, mice, and hamsters.

[4] Mutagenic potency (m) was defined as the reciprocal of the number of micrograms of compound giving 100 revertant colonies in the Ames assay.